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Mechanisms linking metabolic stress with innate immunity in the endometrium[1]

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ABSTRACT

Bacterial infections of the uterus after parturition are ubiquitous in dairy cattle and often cause uterine disease, such as metritis or endometritis. However, the metabolic stress associated with milk production increases the risk of developing disease. Resolution of bacterial infections requires rapid and robust innate immune responses, which depend on host cell receptors recognizing pathogen-associated molecular patterns, such as lipopolysaccharide (LPS) from gram-negative bacteria. Here, we argue that metabolic stress impairs the inflammatory response to pathogens. Glucose and glutamine are the major energy sources for cells, but their abundance is reduced in postpartum dairy cows. Furthermore, inflammatory responses exacerbate metabolic stress, with animals and tissues consuming more glucose when challenged with LPS. However, depriving endometrial tissue of glucose or glutamine impairs the secretion of IL-1β, IL-6, and IL-8 in response to pathogen-associated molecular patterns. Glycolysis and the intracellular sensor of energy, AMP-activated protein kinase, are important for the response to LPS because perturbing glycolysis or AMP-activated protein kinase activity reduces the secretion of IL-1β, IL-6, and IL-8 in the endometrium. The mevalonate pathway for cellular cholesterol synthesis may also be linked to immunity, as inhibition of the terminal enzyme in the pathway, squalene synthase, reduces inflammatory responses to pathogenic bacteria and LPS. In contrast, only modest effects on inflammation are found when modulating the sensor of cellular nutrient satiety, mammalian target of rapamycin, or the endocrine regulator of metabolism, insulin-like growth factor-1. We suggest that stressing cellular metabolism increases the risk of uterine disease by impairing endometrial defenses.

Key words: uterus, bacteria, inflammation, metabolism

INTRODUCTION

Bacterial infections of the uterus are ubiquitous after parturition in dairy cattle, often causing metritis and endometritis (Sheldon et al., 2009). Treating metritis, as well as the subsequent infertility and reduced milk production, costs the European Union and US dairy industries about $2 billion/yr (Sheldon et al., 2009). The risk of developing uterine disease is increased by the metabolic stress associated with milk production (LeBlanc, 2012; Wathes, 2012; Esposito et al., 2014). Countering bacterial infections requires rapid and robust inflammatory responses, driven by the innate immune system (Sheldon et al., 2017). The systems of energy metabolism, innate immunity, and reproduction are highly integrated because they have co-evolved over millennia; thus, stress in one system may affect the others. Here, we argue that cellular metabolic stress impairs the inflammatory response to pathogens in the endometrium.

Many informative epidemiological and whole-animal studies have been carried out on the associations between dairy cow nutrition and disease, particularly in the transition period and early lactation. Reviewing the in vivo studies is beyond the scope of the present paper, and we refer readers to comprehensive reviews that encapsulate this information (Chagas et al., 2007; Sordillo et al., 2009; LeBlanc, 2012; Esposito et al., 2014). Here, we focus on tissue and cells, and review the mechanisms that may link cellular metabolic stress with innate immunity in the endometrium. Evidence is presented for the roles of glucose and glutamine in immunity, and how intracellular sensors of metabolism, hormones, and cholesterol may modulate cellular defense. We suggest that stressing cellular metabolism increases the risk of uterine disease by impairing endometrial defenses.

METABOLIC STRESS

The modern dairy cow is under metabolic stress because the energy demand of lactation is typically 3

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times the resting metabolic rate. For a typical dairy cow producing 40 L of milk/d, the metabolic energy requirements for milk production are about 200 MJ/d, whereas only about 65 MJ/d is needed for maintenance. An equivalent metabolic demand for humans is running 3 marathons every day, because the energy expenditure to complete a marathon is almost the same as the runner's daily energy intake (Williams et al., 1984). Although dairy cattle have been genetically selected to produce milk, they simply cannot consume enough food to meet the metabolic demand of lactation. Consequently, body tissues are broken down to supply metabolic energy and satisfy the negative energy balance (LeBlanc, 2012; Wathes, 2012; Esposito et al., 2014). Negative energy balance is reflected by reduced blood concentrations of glucose, glutamine, and IGF-1 in postpartum animals (Doepel et al., 2006; Chagas et al., 2007; Wathes et al., 2011; Ingvartsen and Moyes, 2013). Furthermore, metabolic stress during early lactation in dairy cattle is associated with an increased incidence of uterine disease (Chagas et al., 2007; Kerestes et al., 2009; Galvão et al., 2010). Even more striking is that reduced appetite before calving increases the risk of severe uterine disease after parturition (Huzzey et al., 2007).

UTERINE DISEASE AND INNATE IMMUNITY

All cows have abundant bacteria in the uterine lumen after parturition (Sheldon et al., 2002). These bacteria come not only from the environment, but may also be part of a resident uterine microflora (Karstrup et al., 2017; Moore et al., 2017). However, high-milk yield cows have a propensity for developing bacterial disease of the endometrium. Up to 40% of animals develop metritis within 10 d of parturition, chronic endometritis persists >3 wk postpartum in about 20% of cows, and subclinical endometritis affects at least an additional 15% of animals (LeBlanc et al., 2002; Sheldon et al., 2002; Kasimanickam et al., 2004; Zwald et al., 2004; Sheldon et al., 2009). Based on culture and molecular techniques, key bacteria in the pathogenesis of postpartum uterine disease include endometrial pathogenic *Escherichia coli*, *Trueperella pyogenes*, and anaerobic bacteria, such as *Fusobacteria*, *Prevotella*, and *Bacteroides* species (Noakes et al., 1991; Sheldon et al., 2002; Sheldon et al., 2010; Amos et al., 2014; Bicalho et al., 2017).

Bacteria are sensed by the innate immune system, with their pathogen-associated molecular patterns detected by pattern recognition receptors, such as Toll-like receptors (**TLR**) on endometrial cells, neutrophils, and macrophages (reviewed in: Sheldon et al., 2017). For example, TLR4 binds the LPS endotoxin of *E. coli*, and TLR2, TLR1, and TLR6 bind bacterial lipopeptides. Activation of TLR signaling in endometrial cells leads to the secretion of prostaglandins and IL-1β, IL-6, and IL-8 (Herath et al., 2006; Herath et al., 2009; Cronin et al., 2012; Turner et al., 2014); however, the response to pathogen molecules is energetically expensive in vivo and in vitro (Turner et al., 2016; Kvidera et al., 2017). A striking example is that animals require an additional kilogram of glucose to supply the activated immune system in the first 12 h after in vivo challenge with LPS (Kvidera et al., 2017). Unfortunately, uterine disease further exacerbates metabolic stress because cows with metritis eat 2 kg/d less food (Wittrock et al., 2011). Metabolic interactions also occur between host and pathogens because tissues and bacteria compete for the same nutrients, and pathogens can sense and adapt to metabolic changes in tissues (Olive and Sassetti, 2016).

Rapid and robust inflammatory responses are important to resist bacterial infections effectively and return animals to health (Medzhitov, 2008; Chovatiya and Medzhitov, 2014; Sheldon et al., 2017). Delayed or inadequate acute inflammatory responses can lead to problems, including failure to clear microbes, disrupting the active resolution of inflammation, and delaying the repair of inflamed and damaged tissues. Missing the opportunity to counter infections effectively often leads to chronic inflammation (Figure 1). Indeed, chronic endometritis is a common sequel to postpartum uterine infections in dairy cows (Chagas et al., 2007; Kerestes et al., 2009; Sheldon et al., 2009; LeBlanc, 2012). Furthermore, animals manipulated to have severe negative energy balance had increased abundance of inflammatory genes in the endometrium 2 wk postpartum, whereas animals in mild negative energy balance appeared to have recovered by that time (Wathes et al., 2009; Wathes, 2012).

Innate immunity is optimally tuned when metabolism is in homeostatic balance (Chovatiya and Medzhitov, 2014; O'Neill et al., 2016). Thus, compromised function of peripheral blood neutrophils may be one explanation for how negative energy balance is linked to uterine disease (Hammon et al., 2006; Galvão et al., 2010; LeBlanc, 2012; Mendonça et al., 2013). However, nutrient availability often has subtle effects on circulating immune cells and considerable variation exists among studies. Furthermore, we reason that the persistence of neutrophils may reflect a failure in the innate immune system to return the postpartum endometrium to homeostasis. Our alternative concept is that stressing cellular metabolism increases the risk of disease by impairing defenses in the tissue and cells of the endometrium.